CLAIMS

WHAT IS CLAIMED IS:

1. A composition comprising:

a translation system;

an orthogonal aminoacyl-tRNA synthetase (O-RS) selected from the group consisting of: an orthogonal tryptophanyl-tRNA synthetase (O-TrpRS), an orthogonal mutant tryptophanyl-tRNA synthetase (O-muTrpRS), and a derivative thereof; and,

an orthogonal tRNA (O-tRNA);

wherein the O-RS preferentially aminoacylates the O-tRNA with an amino acid or unnatural amino acid.

2. The composition of claim 1, wherein the translation system comprises a cell or an *in vitro* translation system.

3. The composition of claim 2, wherein the cell comprises a eukaryotic cell, a *Xenopus* cell, or a mammalian cell.

4. The composition of claim 2, wherein the *in vitro* translation comprises a cell lysate.

5. The composition of claim 1, wherein the O-RS is encoded by a nucleic acid comprising a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 1, a conservative variation thereof, and a complementary polynucleotide sequence thereof.

6. The composition of claim 1, wherein the O-RS comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 2, and a conservative substitution thereof.

7. The composition of claim 1, wherein the unnatural amino acid comprises: a tryptophan analog or 5-hydroxy-L-tryptophan (5-HTPP).

8. The composition of claim 1, wherein the O-RS comprises one or more improved or enhanced enzymatic properties, selected from the group consisting of: Km and Kcat, for the unnatural amino acid as compared to a natural amino acid.

9. The composition of claim 1, wherein the O-tRNA is not substantially aminoacylated by an endogenous aminoacyl-tRNA synthetase of the translation system.

10. The composition of claim 1, wherein the O-tRNA comprises a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 3, a conservative variation thereof, and a complementary polynucleotide sequence thereof.

11. The composition of claim 1, wherein the O-tRNA recognizes a selector codon.

12. The composition of claim 11, wherein the selector codon comprises a sequence selected from the group consisting of: a four base codon, a rare codon, UAG, UAA, and UGA.

13. The composition of claim 1, further comprising a nucleic acid encoding a product peptide.

14. The composition of claim 13, wherein the nucleic acid comprises a selector codon sequence recognized by the O-tRNA.

15. The composition of claim 13, wherein the product peptide comprises an amino acid sequence that is at least 75% identical to that of a wild type therapeutic protein, a diagnostic protein, an industrial enzyme, or a portion thereof.

16. A composition comprising an orthogonal aminoacyl-tRNA synthetase (O-RS), wherein the O-RS preferentially aminoacylates a tRNA with 5-hydroxy-L-tryptophan (5-HTPP).

17. The composition of claim 16, wherein the O-RS is encoded by a nucleic acid comprising a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 1, a conservative variation thereof, and a complementary polynucleotide sequence thereof.

18. The composition of claim 16, wherein the O-RS comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 2, and a conservative substitution thereof.

19. The composition of claim 16, wherein the O-RS comprises one or more improved or enhanced enzymatic properties, selected from the group consisting of: Km and Kcat, for aminoacylation with the 5-HTPP as compared to a tryptophan.

20. The composition of claim 16, wherein the t-RNA is an O-tRNA.

21. The composition of claim 20, wherein the O-tRNA is not substantially aminoacylated by an endogenous aminoacyl-tRNA synthetase.

22. The composition of claim 20, wherein the O-tRNA comprises a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 3, a conservative variation thereof, and a complementary polynucleotide sequence thereof.

23. The composition of claim 20, wherein the O-tRNA recognizes a selector codon.

24. The composition of claim 23, wherein the selector codon comprises a sequence selected from the group consisting of: a four base codon, a rare codon, UAG, UGA, and UAA.

25. The composition of claim 16, further comprising an endogenous translation system.

26. The composition of claim 25, wherein the endogenous translation system comprises a cell or an *in vitro* translation system.

27. The composition of claim 26, wherein the cell comprises eukaryotic cells or mammalian cells.

28. The composition of claim 16, further comprising a nucleic acid encoding a product peptide.

29. The composition of claim 28, wherein the t-RNA is an O-tRNA, and the nucleic acid comprises a selector codon sequence recognized by the O-tRNA.

30. The composition of claim 28, wherein the product peptide comprises an amino acid sequence that is at least 75% identical to that of a wild type therapeutic protein, a diagnostic protein, an industrial enzyme, or a portion thereof.

31. A polypeptide comprising an amino acid sequence encoded by a coding polynucleotide sequence, the coding polynucleotide sequence selected from the group consisting of:

a) a coding polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, and a conservative variation thereof;

b) a coding polynucleotide sequence that encodes a polypeptide selected from the group consisting of SEQ ID NO: 2, and a conservative substitution thereof;

c) a polynucleotide sequence which hybridizes under highly stringent conditions over substantially an entire length of a polynucleotide sequence of (a) or (b);

d) a complementary sequence of (a), (b), or (c); and,

wherein the polypeptide comprises an aminoacyl-tRNA synthetase activity charging with 5-HTPP.

32. A nucleic acid comprising: a polynucleotide sequence selected from the group consisting of:

a) a polynucleotide sequence selected from SEQ ID NO: 3, or a complementary polynucleotide sequence thereof;

b) a conservative variation of (a) that recognizes a selector codon; and,

c) a polynucleotide sequence which hybridizes under highly stringent conditions over substantially the entire length of polynucleotide sequence (a), and which comprises a tRNA that recognizes a selector codon.

33. The nucleic acid of claim 32, wherein the selector codon is selected from the group consisting of: a four base codon, a rare codon, UGA, UAA, and UAG.

34. A method of incorporating an amino acid or unnatural amino acid into a peptide, the method comprising:

preparing a construct comprising a nucleic acid sequence encoding an orthogonal mutant tryptophanyl-tRNA synthetase (O-muTrpRS) or a derivative thereof;

preparing a construct comprising a nucleic acid sequence encoding an orthogonal tRNA (0-tRN^);

introducing the O-muTrpRS construct and the O-tRNA construct into a eukaryotic cell; and,

preferentially aminoacylatiSg an expressed O-tRNA with the amino acid or unnatural amino acid, wherein said aminoacylation is catalyzed by an expressed O-muTrpRS;

whereby the amino acid or unnatural amino acid is incorporated into the peptide in the cell.

35. The method of claim 34, wherein the unnatural amino acid comprises a tryptophan analog or 5-hydroxy-L-tryptophan (5-HTPP).

36. The method of claim 35, further comprising applying a voltage to the peptide, thereby reacting the 5-HTPP with a reactive molecule.

37. The method of claim 36, wherein reacting comprises cross-linking.

38. The method of claim 36, wherein the reactive molecule comprises an unnatural amino acid in another peptide.

39. The method of claim 34, further comprising detecting an interaction between the peptide and another peptide.

40. The method of claim 39, wherein said detecting comprises fluoroscopy.

41. The method of claim 34, wherein the 0-muTrpRS construct comprises a nucleic acid comprising a polynucleotide sequence selected from the group consisting of:

a) a coding polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, and a conservative variation thereof;

b) a coding polynucleotide sequence that encodes a polypeptide selected from the group consisting of SEQ ID NO: 2, and a conservative substitution thereof;

c) a polynucleotide sequence which hybridizes under highly stringent conditions over substantially an entire length of a polynucleotide sequence of (a) or (b);

d) a complementary sequence of (a), (b), or (c); and,

e) pVal144ProBsTr pRS.

42. The method of claim 34, wherein the O-muTrpRS construct comprises a mutated tryptophanyl-tRNA synthetase peptide sequence mutated at one or more amino acid residues based on structure data of the tryptophanyl-tRNA synthetase or an analogous aminoacyl-tRNA synthetase.

43. The method of claim 42, wherein the mutated tryptophanyl-tRNA synthetase comprises a *Bacillus* tryptophanyl-tRNA synthetase mutated at Val144.

44. The method of claim 34, wherein the O-tRNA construct comprises a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 3, a conservative variation thereof, and a complementary polynucleotide sequence thereof.

45. The method of claim 34, wherein said preparing the O-tRNA construct comprises inclusion of one or more tRNA flanking sequences that functionally interact with an RNA polymerase of the cell.

46. The method of claim 34, wherein the O-tRNA construct comprises an A box eukaryotic transcriptional control element.

47. The method of claim 34, further comprising mutating a prokaryotic tRNA sequence to include a functional A box eukaryotic transcriptional control element.

48. The method of claim 47, wherein said mutating comprises site directed mutagenesis.

49. The method of claim 34, wherein the O-tRNA construct or O-muTrpRS construct comprises: a reporter tag or a purification tag.

50. The method of claim 34, wherein the O-muTrpRS construct and the O-tRNA construct comprise the same construct.

51. The method of claim 34, wherein the O-tRNA recognizes a selector codon in a nucleic acid sequence encoding the peptide, thereby incorporating the unnatural amino acid into the peptide.

52. The method of claim 34, further comprising transfecting a nucleic acid encoding the peptide into the cell.

53. The method of claim 52, wherein the cell comprises a eukaryotic cell or mammalian cell.

54. The method of claim 34, further comprising expressing the O-muTrpRS construct or the O-tRNA construct.

55. The method of claim 54, further comprising purifying expressed O-muTrpRS or expressed O-tRNA.

56. A mammalian cell comprising:

an orthogonal aminoacyl-tRNA synthetase (O-RS) selected from the group consisting of: an orthogonal tryptophanyl-tRNA synthetase (O-TrpRS), an orthogonal mutant tryptophanyl-tRNA synthetase (O-muTrpRS), and a derivative thereof; and,

an orthogonal tRNA (O-tRNA);

wherein the O-RS preferentially aminoacylates the O-tRNA with an amino acid or unnatural amino acid.

57. The mammalian cell of claim 56, wherein the O-RS comprises a nucleic acid comprising a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 1, a conservative variation thereof, and a complementary polynucleotide sequence thereof.

58. The mammalian cell of claim 56, wherein the O-RS comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 2, and a conservative substitution thereof.

59. The mammalian cell of claim 56, wherein the O-tRNA is not substantially aminoacylated by an endogenous aminoacyl-tRNA synthetase of the cell.

60. The mammalian cell of claim 56, wherein the O-tRNA comprises a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 3, a conservative variation thereof, and a complementary polynucleotide sequence thereof.

61. The mammalian cell of claim 56, wherein the unnatural amino acid comprises an amino acid selected from the group consisting of: a tryptophan analog and 5-hydroxy-L-tryptophan (5-HTPP).